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TOXICOKINETICS AND TOXICODYNAMICS OF PYRETHROID INSECTICIDES IN FISH

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Abstract—The pyrethroid insecticides are extremely toxic to fish, with 96-h LC50 values generally below 10 μg/L and i.p. and i.v. LD50 values below 20 mg/kg. Corresponding LD50 values in mammals and birds are in the range of several hundred to several thousand milligrams per kilogram. This review examines pyrethroid toxicokinetics and toxicodynamics in fish as critical factors associated with species selectivity. Studies with permethrin, cypermethrin and fenvalerate have established that rates of metabolism and elimination in rainbow trout are significantly lower than those reported for birds and mammals. Comparatively low lethal brain pyrethroid concentrations and nonneural aspects of pyrethroid intoxication in fish suggest that variations in toxicodynamics are also crucial in evaluating pyrethroid selectivity.

Keywords—Pyrethroid insecticides Fish Toxicokinetics Toxicodynamics

INTRODUCTION

The pyrethroid insecticides are extremely toxic to fish during aqueous exposures, with 96-h LC50 values generally below 10 μg/L [1–3]. In contrast, mammals and birds are generally very tolerant to p.o. and i.p. pyrethroid administration, with LD50 values in the range of several hundred to several thousand milligams per kilogram. Comparisons of mammalian LD50 values with fish LC50 values are, however, difficult to interpret. Recently, several researchers have reported permethrin (3-phenoxybenzyl [*R,S*]-*cis,trans*-3-[2,2-dichlorovinyl]-2,2-dimethylcyclopropanecarboxylate) and fenvalerate ([*R,S*]-α-cyano-3-phenoxybenzyl [*R,S*]-2-[4-chlorophenyl]-3-methylbutyrate) toxicity values in rainbow trout (*Salmo gairdneri*) and bluegill (*Lepomis macrochirus*) after administration by the routes used in mammalian studies (Table 1). An analysis of these studies reflects a one- to three-order-of-magnitude greater toxicity for pyrethroids in fish as opposed to mammals.

The extreme susceptibility of fish to the pyrethroids and the noted vertebrate selectivity are seemingly an outcome of toxicokinetic (i.e., absorption, biotransformation, distribution, elimination) and toxicodynamic (i.e., biochemical/physiological effects) factors. This review critically examines the influence of these parameters on the aquatic toxicology of the synthetic pyrethroids.

TOXICOKINETICS

Bradbury et al. [4] studied the toxicokinetics of [aromatic-^{3}H]fenvalerate in rainbow trout using specially designed flow-through metabolism-respirometer chambers that permitted a determination of gill uptake and elimination of insecticide, as well as urinary and biliary excretion. Fish were exposed to fenvalerate at concentrations of 20 and 0.3 ng/L for 36 to 48 h. Depuration of the radiolabel was monitored for 8 to 48 h. A relatively inefficient fenvalerate gill uptake of 28% was consistent with the passive diffusion model of xenobiotic absorption developed by McKim et al. [5]. These results suggested that at log *P* values between 6 and 7 (typical for pyrethroids [6]), gill

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The analyses and conclusions herein are those of the authors and do not necessarily reflect the views of the U.S. Environmental Protection Agency; hence, no agency endorsement should be inferred.

Table 1. Acute lethality of permethrin and fenvalerate to mammals and fish

Pyrethroid	Route of exposure	LD50 (mg/kg) Rainbow trout	Bluegill	Mouse
Permethrin	i.p.	14[a]	NT	514[a]
(technical)	i.v.	1.8[b]	NT	31[b]
cis-Permethrin	i.p.	22[a]	NT	108[a]
	i.v.	1.3[b]	NT	17[b]
trans-Permethrin	i.p.	7[a]	NT	>800[a]
	i.v.	1.2[b]	NT	>135[b]
Fenvalerate				
2*R*,*S*,α*R*,*S* (technical)	i.p./p.o.	NT	0.67[c]	245,[d] >500[e]
2*S*,α*S*	i.p./p.o.	NT	0.12[c]	50[d]
2*S*,α*R*	i.p./p.o.	NT	11.6 [c]	NT
2*S*,α*R*,*S*	i.p./p.o.	NT	NT	>600[d]
2*R*,α*S*	i.p./p.o.	NT	>212[c]	NT
2*R*,α*R*	i.p./p.o.	NT	>212[c]	NT
2*R*,α*R*,*S*	i.p./p.o.	NT	NT	>5,000[d]

NT, not tested.
[a]From ref. 11.
[b]From ref. 12.
[c]From ref. 22 (i.p. exposure).
[d]From ref. 46 (p.o. exposure).
[e]From ref. 47 (p.o. exposure).

uptake efficiency drops from 60% (at log *P* values of 3 to 6) to about 20 to 30%. It had previously been postulated that, because of their lipophilicity, pyrethroids would have a high rate of gill absorption, which, in turn, would be a contributing factor in the sensitivity of fish to aqueous pyrethroid exposures [6]. The results of this study, however, suggest that efficient gill absorption probably is not a contributing factor in pyrethroid toxicity.

After the 8- to 48-h depuration periods, carcass and bile contained 80 to 90% and 10 to 20% of the gill-absorbed doses, respectively. No radiolabel was eliminated across the gills. Biliary analysis revealed that the glucuronide of 4′-hydroxyfenvalerate was the only major metabolic product (Fig. 1). Significant levels of ester hydrolysis products were not found in urine or bile. Urine, feces and blood each contained less than 2% of the dose. Within 8 to 12 h after exposure, significant urinary excretion and blood transport were completed. Tissues

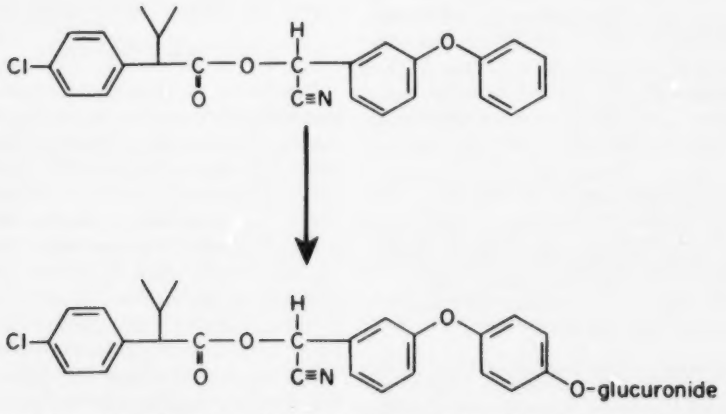

Fig. 1. In vivo biotransformation of fenvalerate in rainbow trout, based on an analysis of bile metabolites [4].

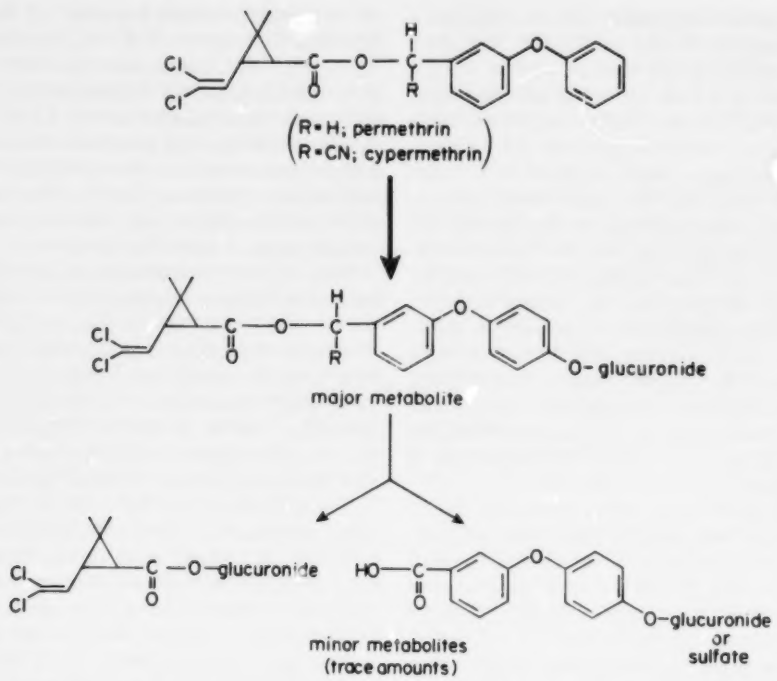

Fig. 2. In vivo biotransformation of permethrin [11] and cypermethrin [17] in rainbow trout, based on an analysis of bile and urine metabolites.

from trout exposed to 0.3 ng/L fenvalerate were also analyzed for fenvalerate equivalents. Fat contained the highest concentrations (about 200 pg/g). The remaining tissues contained between 15 and 45 pg/g. The findings in rainbow trout contrast to those reported for rats [7] and quail [8,9], in which 90 to 100% of administered fenvalerate was eliminated within 48 h. Ester hydrolysis, the major degradation reaction in mammals [7,10] and birds [8,9], was not observed in trout.

Glickman and coworkers [11–14] studied the metabolism of permethrin in rainbow trout extensively. In vivo studies with [carbonyl-^{14}C]- and [methylene-^{14}C]*cis*- and *trans*-permethrin [11] indicated rates of elimination and metabolism generally slower than those reported in mammals [15] and birds [16]. In trout, no substantial difference in the toxicokinetics of the cis and trans isomers was noted. After aqueous exposure of trout to 5 μg/L permethrin, approximate half-lives in fat, brain, liver, muscle and blood were greater than 168, 15, 30, 26 and 17 h, respectively. Radiocarbon tended to concentrate in fat, with residues generally 20 to 30 times higher than those in blood 48 h after aqueous exposure. Forty-eight hours after an i.p. exposure (0.5 mg/kg), 30 to 40% of the dose remained in the carcass. An additional 30 to 40% of the dose was recovered in the bile, whereas only 3 to 7% of the dose was eliminated in the urine. Analysis of bile for metabolites 48 h postdose established that the sole product was the glucuronide of 4′-hydroxypermethrin (Fig. 2). Radioactivity in the urine seemed to be associated with ester hydrolysis products; however, low activity made identification impossible. The results of this study suggest that permethrin metabolism and elimination are substantially slower and qualitatively different from that reported in mammals and birds [15,16], with oxidative degradation predominating and ester hydrolysis constituting a minor reaction. However, greater rates of elimination and metabolism of permethrin, as compared with fenvalerate, in trout may be a contributing factor to its lower toxicity to fish (see refs. 1–3).

Although earlier in vitro studies indicated that rainbow trout could metabolize permethrin in a

manner qualitatively similar to that of mammals [13], subsequent studies established that trout had comparatively low esterase activity toward permethrin. In a study with trout and mice tissue preparations, incubated at physiologically relevant temperatures, Glickman and Lech [14] reported that the hydrolysis of *trans*-permethrin on a whole-body basis was about 184 times slower in trout. Mouse liver, kidney and plasma preparations hydrolyzed permethrin 166, 38 and 59 times faster, respectively, than corresponding trout tissues. By using tri-*o*-tolyl-phosphate, an esterase inhibitor, and measuring potentiation of permethrin lethality, it was established that ester hydrolysis was a significant detoxification route in mice, whereas esterase inhibition in trout had little effect on toxicity [12]. Glickman et al. [12] also established that microsomal oxidation of *trans*-permethrin was 35 times slower in trout preparations.

The results of studies with cypermethrin ([*R,S*]-α-cyano-3-phenoxybenzyl [*R,S*]-*cis,trans*-3-[2,2-dichlorovinyl]-2,2-dimethylcyclopropanecarboxylate) in rainbow trout [17] are similar to those reported for permethrin. After aqueous exposure (5 μg/L) to [cyclopropyl-^{14}C]- or [benzyl-^{14}C]*cis*-cypermethrin, 27% of the dose was recovered in the bile by 24 h postdose. The glucuronide of 4′-hydroxycypermethrin was the major biliary metabolite recovered (Fig. 2). Minor levels of ester hydrolysis products were also recovered. In vitro studies of cypermethrin metabolism by trout liver preparations indicated a 10- to 20-fold lower level of enzymatic activity (routes not specified) as compared with the mouse and Japanese quail (*Coturnix coturnix*) [17].

An analysis of the literature clearly demonstrates that toxicokinetic factors play an important role in the sensitivity of fish to pyrethroid intoxication. Studies with fenvalerate suggest that efficient gill uptake does not explain the susceptibility of fish to pyrethroids during aqueous exposures [4]. Although gill uptake of pyrethroid may be inefficient relative to that of other xenobiotics, direct uptake into the bloodstream is an important consideration. The route of pyrethroid administration in mammals does influence toxicity; fenvalerate is more lethal to rats after an i.v. exposure (50 to 100 mg/kg) [18] than after p.o. administration (450 mg/kg) [19]. Similar trends in permethrin toxicity have been noted in mice as well [11,12] (Table 1). Increased toxicity with i.v. administration, as compared with p.o. and i.p. dosing, is generally attributed to reduced levels of detoxification because of the elimination of first-pass metabolism in the liver. Lethal aqueous exposures to fenvalerate in fathead minnows [20] and rainbow trout [21] result in whole-body doses of 0.2 to 1.5 mg/kg, which are similar to i.p. LD50 values in bluegill [22] (Table 1). These values are substantially lower than mammalian i.v. LD50 values. Comparable dosing with permethrin indicates that, after i.v. administration, trout are still significantly more sensitive than mice (Table 1). Thus, the route of pyrethroid exposure does not seem to be a significant factor in pyrethroid selectivity.

Low rates of elimination and metabolism do seem to be critical in assessing the susceptibility of fish to pyrethroid intoxication. Half-lives for elimination of pyrethroids in trout are in excess of 48 h [4,11,17], whereas in mammals and birds, the half-lives are in the range of 6 to 12 h [7–10,15,16]. Generally, only 20 to 30% of the administered dose is metabolized by trout [4,11,17]. In terrestrial vertebrates, usually 90% of the pyrethroid dose is metabolized, with up to 70% of the metabolites associated with ester hydrolysis products [7–10,15,16]. In general, trout exhibit little or no esterase activity toward pyrethroids and substantially lower oxidative activity, as compared with mammals and birds. Studies with mixed function oxidase and esterase inhibitors in the mouse and trout confirm the role of detoxification (especially ester hydrolysis) in species selectivity [12]. Interestingly, the carp (*Cyprinus carpio*), which is somewhat less sensitive to the pyrethroids than trout [2], seems to have higher esterase activity toward *trans*-permethrin [13] and fenvalerate [23,24]. Although fish metabolize and eliminate pyrethroids at lower rates than birds and mammals, these rates are seemingly sufficient to minimize significant bioconcentration (e.g., bioconcentration factors for fenvalerate of several hundred to about 3,000 [20,22,23,25]).

TOXICODYNAMICS

It is readily accepted that the acute mode of action of the pyrethroids is a function of their neuroactivity. Hypotheses regarding pyrethroid mode of action in the nervous system are reviewed by Clark and Brooks [26] in this issue. Reports on the toxic responses of fish to pyrethroid intoxication are addressed here, with special reference to the implications of possible nonneural modes of pyrethroid action.

As reviewed by Gray and Soderlund [27], the effect of pyrethroids in mammals is highly stereospecific; only esters of 1*R* cyclopropanecarboxylates and 2*S* isomers of noncyclopropane acids are

toxic. In addition, a cis configuration across the cyclopropane ring imparts greater toxicity than the corresponding trans isomers. An alpha cyano substituent in an *S* configuration at the benzylic position of the 3-phenoxybenzyl alcohol moiety also greatly enhances neurotoxicity. Addition of the alpha cyano group to nontoxic *trans*-cyclopropanecarboxylates can result in potent neurotoxicants (e.g., *trans*-permethrin to *trans*-cypermethrin). Structure–toxicity relationships in insects indicate less specific isomeric requirements for toxicity [6,28,29], where *trans*-cyclopropanecarboxylate and [*R*]α-cyano-3-phenoxybenzyl alcohol esters maintain comparable, or at least limited, activity.

The data available for fish suggest qualitatively intermediate isomeric responses, in comparison to those of invertebrates and mammals. Whereas mammals show differential responses to *cis*- and *trans*-cyclopropanecarboxylate esters, fish seem to be equally sensitive to both cis and trans isomers of permethrin (Tables 1 and 2). In contrast, the response of fish to the enantiomers of fenvalerate suggests a stereospecific relationship similar to that of mammals (Table 1), with toxicity primarily dependent on the 2*S*,α*S* component of the technical mixture [22].

Interpretation of pyrethroid poisoning signs in mammals and terrestrial insects has led to the classification of Type I (or T) and Type II (or CS) syndromes [18,30–32]. In mammals (primarily rats and mice), Type I signs include aggressive sparring behavior, increased sensitivity to external stimuli, fine tremors, prostration, coarse body tremor and an increase in body temperature. Generally, non-alpha cyano pyrethroids elicit the Type I syndrome. Type II symptoms include chewing, profuse salivation, pawing and burrowing, coarse body tremor, increased startle response, abnormal locomotion of the hind limbs, sinuous writhing (choreoathetosis) and clonic and tonic seizures. Pyrethroids associated with the Type II syndrome generally contain an *S*-α-cyano-3-phenoxybenzyl alcohol moiety. In the cockroach, Type I signs include restlessness, incoordination, prostration and paralysis, whereas Type II signs include incoordination, convulsions and intense hyperactivity.

An examination of pyrethroid intoxication in the frog also results in a similar classification of pyrethroid symptoms [33]. Type I pyrethroids (non-alpha cyano) cause incoordination (extension and lack of control of the hind limbs and ineffective use of the front legs), hyperactivity and tremors, and no tonic seizures. The Type II syndrome, associated with alpha cyano pyrethroids, includes tonic extensions of the limbs, choreoathetosis and salivation.

Detailed reports of toxic signs in fish are limited to only a few compounds, making it difficult to differentiate between Type I and Type II syndromes, as has been done for insects and mammals. Permethrin intoxication in rainbow trout [11,34] and fathead minnows [34]; fenvalerate intoxication in rainbow trout [21,34], fathead minnows [20,34] and bluegill [22]; and cypermethrin intoxication in rainbow trout [35] result in generally similar signs. Fathead minnows initially show a loss of schooling behavior and swim near the water's surface. This is followed by hyperactivity, erratic swimming and violent whole-body contractions (seizures) and a loss of buoyancy and body trim. The effects in rainbow trout are similar, except that flaring of the gill arches and head shaking (tonic seizures [21]) are noted as well. Increased cough rate was also noted by Holcombe et al. [34] and Bradbury et al. [21] during permethrin and fenvalerate intoxication, respectively. Fish generally become inactive before death. Differentiation of toxic signs between pyrethroid classes

Table 2. Lethal brain concentrations (μg/g) of pyrethroids

Pyrethroid	Mouse	Quail	Rainbow trout	Frog (*Rana temporaria*)
cis-Cypermethrin	1.7[a]	4.0[a]	0.2[a]	0.08[a]
Fenvalerate	1.0[b]	1.3[c]	0.16[d]	NT
cis-Permethrin	6.0[e]	NT	2.0[e]	NT
trans-Permethrin	36.4[e]	NT	2.0[e]	NT

NT, not tested.
[a]From ref. 35 (for the 1*R*,α*S* isomer only). Aqueous exposures to trout and frog and p.o. exposures to the mouse and Japanese quail.
[b]From ref. 32 (intracerebral LC50 value for the 2*S*,α*S* isomer).
[c]From ref. 48 (p.o. exposure to bobwhite quail, *Colinus virginianus*).
[d]From ref. 21 (aqueous exposure).
[e]From ref. 49 (i.v. and i.p. exposures).

in fish requires systematic testing of additional compounds and the implementation of standard observational techniques (e.g., see ref. 36).

Recently, the physiological response of rainbow trout to acute fenvalerate intoxication was reported [21]. Fish exhibited initial hyperexcitability, significantly elevated cough rate and gill mucous secretion, and, eventually, tonic seizures, as described previously. A cessation of ventilatory and cardiac activity was noted during seizures. Interpretation of respiratory-cardiovascular and blood-chemistry responses indicated an elevated rate of anaerobic metabolism associated with increasingly severe seizures; i.e., as activity intensified, arterial oxygen, carbon dioxide and pH dropped. These responses in cardiovascular and blood-chemistry variables were similar to those reported after deltamethrin ([1*R*,3*R*]-3-[2,2-dibromovinyl]-2,2-dimethylcyclopropanecarboxylate) intoxication in the rat [37,38] and were correlated with central nervous system activity [37].

In addition to nervous system involvement, the findings also suggested that fenvalerate may adversely affect gill structure. Histopathological examination of the gill tissue revealed lamellar aneurysms and necrotic pillar cells. Filamental tissue between adjacent lamellae was commonly separated from the connective tissue that forms the central sinus of the filament. Numerous necrotic cells were also found in these separated interlamellar zones. Similar types of pathology have been reported after subacute exposure of rainbow trout to permethrin as well [39]. In both studies, damage to gill surfaces was attributed to irritation and presumably was related to evaluated mucous secretion, increased ventilation volume and decreased gill oxygen-uptake efficiency in fenvalerate-intoxicated trout. Increased cough rate may have been a function of the morphological changes in the gill or may have been due to interactions of fenvalerate with receptors on the walls of the pharynx and gill arches. (Fenvalerate has been reported to cause skin paresthesias in humans, seemingly due to effects on sensory nerves [40,41].)

Finally, urine osmolality and urinary Na^+ and K^+ excretion rates and concentrations were significantly elevated in intoxicated trout, which suggests that fenvalerate may interfere with renal ion regulation. Interactions between fenvalerate intoxication and osmoregulation has been suggested in the estuarine grass shrimp as well [42]. Numerous studies have established that the pyrethroids are capable of inhibiting a number of ATPase-utilizing systems [43–45]. Inhibition of mitochondrial ATPase and/or the Na^+-K^+ pump in the kidneys could be responsible for the observed increases in Na^+ loss from trout. Dropping Na^+ concentrations in the body could potentiate the neurological effects associated with fenvalerate intoxication. The findings of this study suggest that effects on respiratory structures and renal ion regulation, in addition to effects on the nervous system, may be important factors in assessing the toxicodynamics of pyrethroid insecticides in fish.

Undoubtedly, pyrethroid selectivity among the vertebrates is at least partly related to detoxification, but differences at the site(s) of action may also be significant. When pyrethroid metabolism is inhibited, *trans*-permethrin is still 65 times more toxic to the trout than to the mouse [12]. A comparison of brain pyrethroid concentrations at death, across a wide range of organisms (Table 2), indicates large differences among birds and mammals and aquatic species. On the basis of lethal brain concentrations, the rainbow trout and frog are approximately one order of magnitude more sensitive to pyrethroids than the mouse or quail, which suggests possible differences in neurosensitivity. Alternatively, these differences suggest that additional nonneural sites of action are a factor in the toxicodynamics of pyrethroids in aquatic vertebrates. Further evaluation of species differences requires the development of assays specific to the receptor or biochemical process associated with the pyrethroid mode(s) of action.

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